

REMARKS

Applicants would like to thank Examiner Robert Zeman for the very courteous and helpful discussion held with Applicants's representative on February 17, 2005. During the interview, it was noted that conventional methods for measuring malaria parasites by flow cytometry—such as the methods described in *Saito-Ito et al. (Parasitology International 2001, 50, 249-257)*—involve hemolysis of erythrocytes using only a single surfactant to release malaria parasite. As described in the specification (e.g., page 5, lines 11-17; page 10, lines 2-9; page 32, line 10 to page 34, line 22), these methods suffer from a serious drawback in that reticulocytes that have not undergone sufficient hemolysis appear in a region of a scattergram that overlaps with a region in which malaria parasites to be detected are located.

In contrast to the above-described conventional methods, the claimed invention provides methods wherein a specimen is treated with a first hemolytic agent comprising a first surfactant and a second hemolytic agent comprising a second surfactant having a hemolytic activity higher than that of the first surfactant. By conducting the hemolysis of an erythrocyte in two steps, the effect of reticulocyte may be eliminated to enable more accurate detection of malaria parasite (e.g., specification, page 10, lines 9-14).

During the interview, the chelating agent EDTA was discussed. It was noted that the EDTA used to supplement blood samples in *Saito-Ito et al.* is an anticoagulant—not a surfactant in the sense of the claimed invention. The role of EDTA as an anticoagulant in the methods described by *Saito-Ito et al.* is further confirmed by the paragraph bridging pages 250-251. As is evident from this description, blood samples collected from a patient were pre-treated with an EDTA anticoagulant to ready them for storage at 4 °C. These treated samples were not assayed by flow cytometry until the following day, at which time red blood cells in the sample were counted. The fact that these cells could be counted confirms that the EDTA added to the sample functions as an anticoagulant.

It was further noted in the discussion that just because detergents and emulsifiers may be used as surfactants and the chelating agent EDTA may be added to detergents and emulsions does not necessarily mean that EDTA itself is a surfactant in

the sense of the claimed invention. Moreover, it was also noted that according to the *Hawley's* reference cited in the Office Action (*Hawley's Condensed Chemical Dictionary*, 14th Edition, 2002, John Wiley & Sons, Inc.), EDTA is described for use in detergents (i.e., not as a detergent *per se*) and as a metal deactivator for use in oil emulsions (i.e., not as an emulsifier *per se*).

Claim Rejections – 35 U.S.C. §§ 102(b) and 103(a)

The rejection of claims 1-9 and 23-35 under 35 U.S.C. § 102(b) as being anticipated by or, in the alternative, under 35 U.S.C. § 103(a) as being obvious over *Saito-Ito et al.* has been obviated.

Each of independent claims 1 and 5 has been clarified to recite that the sample or specimen to be tested has been treated with an anticoagulant. Thus, as presently written, independent claims 1 and 5 require treating a specimen or sample that has been treated with an anticoagulant with a first hemolytic agent comprising a first surfactant, and a second hemolytic agent comprising a second surfactant having a hemolytic activity higher than that of the first surfactant. By contrast, *Saito-Ito et al.* only describes treating a specimen with an anticoagulant (i.e., EDTA) and hemolyzing erythrocytes using only a single surfactant (i.e., dodecyl methyl ammonium chloride) to release malaria parasite. *Saito-Ito et al.* does not teach or suggest treatment with a second hemolytic agent comprising a second surfactant having a hemolytic activity higher than that of the first surfactant.

In view of the clarifying amendment to independent claims 1 and 5, there is no way in which to construe the EDTA anticoagulant described by *Saito-Ito et al.* as being a second surfactant in the sense of the claimed invention, even for the sake of argument. Either the EDTA is an anticoagulant, as Applicants maintain, or a surfactant, as was previously argued by the Examiner; however, this one ingredient cannot simultaneously satisfy two recited elements of the independent claims, which presently recite an anticoagulant, a first hemolytic agent comprising a first surfactant, and a second hemolytic agent comprising a second surfactant having a hemolytic activity higher than that of the first surfactant.

Further proof that the methods described in *Saito-Ito et al.* do not involve treatment with a second hemolytic agent comprising a second surfactant having a hemolytic activity higher than that of the first surfactant is evident when the results of the *Saito-Ito et al.* method are compared to those of the claimed invention.

Saito-Ito et al. describes a flow cytometric system for the detection of *Plasmodium falciparum* that involves lysing erythrocytes and staining parasites simultaneously using a hemolysing and staining solution containing dodecyl methyl ammonium chloride and acridine orange (e.g., page 249, Abstract, lines 1-3; page 250, col. 1, last two sentences of Introduction). The methods described in *Saito-Ito et al.* are very clearly affected by residual reticulocytes, which greatly compromise the clinical utility of the methods described. For example, *Saito-Ito et al.* acknowledges that reticulocyte remaining in the samples obscures the measurement region of the malaria parasites to be detected. Thus, *Saito-Ito et al.* states that the methods described therein are “difficult to apply ... to clinical diagnosis” because “particles that seemed to be residual bodies of nuclei remained and were plotted in the parasite area of the scattergram” (e.g., *Saito-Ito et al.*, page 256, columns 1 and 2). As explained below, this admission is further corroborated by the data in the specification.

As noted in *Saito-Ito et al.* (e.g., page 257, second sentence under Acknowledgements), the single-surfactant methods described therein are the subject of Japanese published unexamined patent application No. 11-75892. The single-surfactant methods of *Saito-Ito et al.* and of Japanese application No. 11-75892 are described in the specification and contrasted with the claimed invention (e.g., page 6, lines 7-20; page 10, lines 2-14; Comparative Examples 1-3, page 32, line 10 to page 40, line 6). For example, as described in the specification (e.g., page 10, lines 2-14, emphases added):

It was found that that when the methods described by A. Saito-Ito (*Parasitol. Int.*) and in Japanese Unexamined Patent Publication No. 11-75892 were applied to the measurement of blood from an actual malaria-infected patient, reticulocyte still exists that has not undergone sufficient hemolysis, thereby resulting in increased background which makes it difficult to conduct correct

measurements of the specimen. However, in accordance with the present invention, it was discovered that by conducting the hemolysis of an erythrocyte in two steps, the effect of reticulocyte can be fully eliminated, thereby enabling correct and highly accurate detection of the malaria parasite.

Moreover, as described in Comparative Example 1 of the specification, when only a single surfactant was used to treat a specimen as per the methods of *Saito-Ito et al.*, a large number of reticulocytes did not undergo sufficient hemolysis; these reticulocytes are clearly shown in the dotted-line oval region of FIG. 2 (e.g., page 33, lines 3-5). As a result, false detection of malaria parasites in non-malaria specimens and reductions in measurement sensitivity may result (e.g., specification, page 5, lines 11-17; page 33, lines 6-10). Moreover, attempts to correct this problem by increasing surfactant concentration in an effort to promote reticulocyte hemolysis failed because the increased surfactant concentrations also promoted dissolution of malaria parasite (e.g., specification, page 33, line 18 to page 34, line 22).

As clearly demonstrated by Example 1 and Comparative Examples 1-3 of the specification (page 30, line 20 to page 40, line 6), the methods of the claimed invention whereby a specimen is treated with a first hemolytic agent comprising a first surfactant and a second hemolytic agent comprising a second surfactant having a hemolytic activity higher than that of the first surfactant enable sufficient hemolysis of erythrocytes and reticulocytes in a sample without causing damage to malaria parasites that would affect measurement (e.g., specification, page 40, lines 1-6).

Inasmuch as *Saito-Ito et al.* neither teaches or suggests a second hemolytic agent comprising a second surfactant having a hemolytic activity higher than that of the first surfactant, Applicants respectfully submit that the claimed invention is neither anticipated by nor would have been obvious in view of *Saito-Ito et al.* Accordingly, withdrawal of this ground of rejection is respectfully requested.

New Claims

New dependent claims 36-37 recite different types of anticoagulants that are described in the specification.

Inasmuch as independent claims 1 and 5 are neither anticipated by nor would have been obvious in view of *Saito-Ito et al.* for at least the reasons set forth above, Applicants respectfully submit that new dependent claims 36 and 37, which depend from independent claims 1 and 5, respectively, are likewise neither anticipated by nor would have been obvious in view of *Saito-Ito et al.*

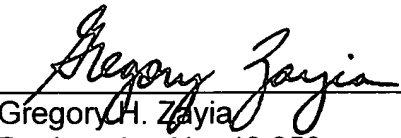
Accordingly, Applicants respectfully submit that new claims 36-37 are in condition for allowance as presently written.

Conclusion

In view of the Amendment and Remarks set forth above, Applicants respectfully submit that the claimed invention is in condition for allowance. Early notification to such effect is earnestly solicited.

If for any reason the Examiner feels that the above Amendment and Remarks do not put the claims in condition to be allowed, and that a discussion would be helpful, it is respectfully requested that the Examiner contact the undersigned agent directly at (312)-321-4257.

Respectfully submitted,



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